Vitamin D and type 1 diabetes mellitus: state of the art

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Recent evidence suggests a role for vitamin D in pathogenesis and prevention of diabetes mellitus. Active vitamin D, $1\alpha,25(OH)_2D_3$, prevents type 1 diabetes in animal models, modifies T-cell differentiation, modulates dendritic cell action and induces cytokine secretion, shifting the balance to regulatory T cells. High-dose vitamin D supplementation early in life protects against type 1 diabetes. $1\alpha,25(OH)_2D_3$ activity is mediated through its receptor, and targets include transcriptional regulators; therefore, $1\alpha,25(OH)_2D_3$ influences gene transcription. $1\alpha,25(OH)_2D_3$ also affects pancreatic β-cell function. Genomic variations of vitamin D metabolism and target cell action predispose to type 1 diabetes. Vitamin D deficiency in pregnancy probably increases the incidence of autoimmune diseases, such as type 1 diabetes, in genetically predisposed individuals. Pharmacotherapy with $1\alpha,25(OH)_2D_3$ analogues might help prevent and treat diabetes.

Introduction

Vitamin D can be taken up from food (e.g. fatty fishes and their oils), but most people achieve their vitamin D needs through direct ultraviolet B (UVB)-mediated synthesis in the skin. Two hydroxylation steps are needed to activate vitamin D, one in the liver (by the enzyme 25-hydroxylase, or CYP2R1, leading to 25-hydroxyvitamin D_3-25-OHD$_3$) and a second in the kidney, leading to the active secosteroid hormone, $1\alpha,25$-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$] [1]. This final hydroxylation (carried out by the enzyme 1-α hydroxylase or CYB27B1) can also occur in several cells outside the kidney, allowing the paracrine secretion of $1\alpha,25(OH)_2D_3$ in other tissues, such as sites of inflammation, where activated macrophages are important sources of the active vitamin D metabolite [2] (Figure 1). Vitamin D and its metabolites are transported in the circulation by vitamin D-binding protein and the complex enters the cell together with megalin and cubilin, recently characterized carrier proteins [3]. Vitamin D exerts its actions in a variety of cell types by binding to the nuclear vitamin D receptor (VDR), which shares its structure with many other nuclear steroid hormone receptors, such as the glucocorticoid, thyroid hormone and estrogen receptors.

This review presents the currently available data on a potential link between vitamin D, its metabolites and receptor system with type 1 diabetes. The effects of vitamin D on the immune system and on pancreatic β cells are reviewed and the implications for type 2 diabetes are discussed.

Environmental sources of vitamin D

Vitamin D concentrations in the blood depend on sun exposure and alimentary intake. Levels of $1\alpha,25(OH)_2D_3$ are also determined by the activity of the enzymes responsible for its final hydroxylation (CYP27B1) and catabolism (mainly CYP24). The vitamin D status is usually assessed by measuring 25-OHD$_3$ levels in the blood. Vitamin D serum concentrations are low or overtly deficient in a sizeable proportion of studied populations and decrease with age. Therefore, routine supplementation is advised, particularly for those at risk of or suffering from osteoporosis, and also in other conditions, such as pregnancy, lactation and early childhood [4]. Optimal dosing is still a matter of debate, but it appears that the current recommendation of 200–400 U d^{-1} (5–10 μg d^{-1}) is not sufficient, and doses below 1000 U d^{-1} will not raise 25-OHD$_3$ levels to 15–80 ng ml^{-1} [5], currently thought to be normal. Moreover, in recent years new data suggest that the physiological range of 25-OHD$_3$ should be raised to even higher levels, bringing the target zone to between 30 and 80 ng ml^{-1} [6,7], although the official bodies have not yet accepted these new ranges. To reach these higher vitamin D concentrations [5], even higher doses of vitamin D would be required (3000–5000 U d^{-1}) [8,9].

Genetics of the vitamin D system

Vitamin D deficiency often runs in families, suggesting that genetic variation might account for differences in vitamin D concentrations; however, the genes regulating vitamin D concentrations remain to be identified.

Genetic variation occurs in nearly all genes of the vitamin D system, but most investigations have been performed for the VDR. The *VDR* gene spans nearly 100 kb on chromosome 12q12–14. The *CYB27B1* gene is also found on chromosome 12, at 12q.13.1–13.3, 10 Mb centromeric of the *VDR* gene. Whereas mutations in *CYP27B1* cause vitamin D-dependent rickets, polymorphisms of the gene are associated with type 1 diabetes,

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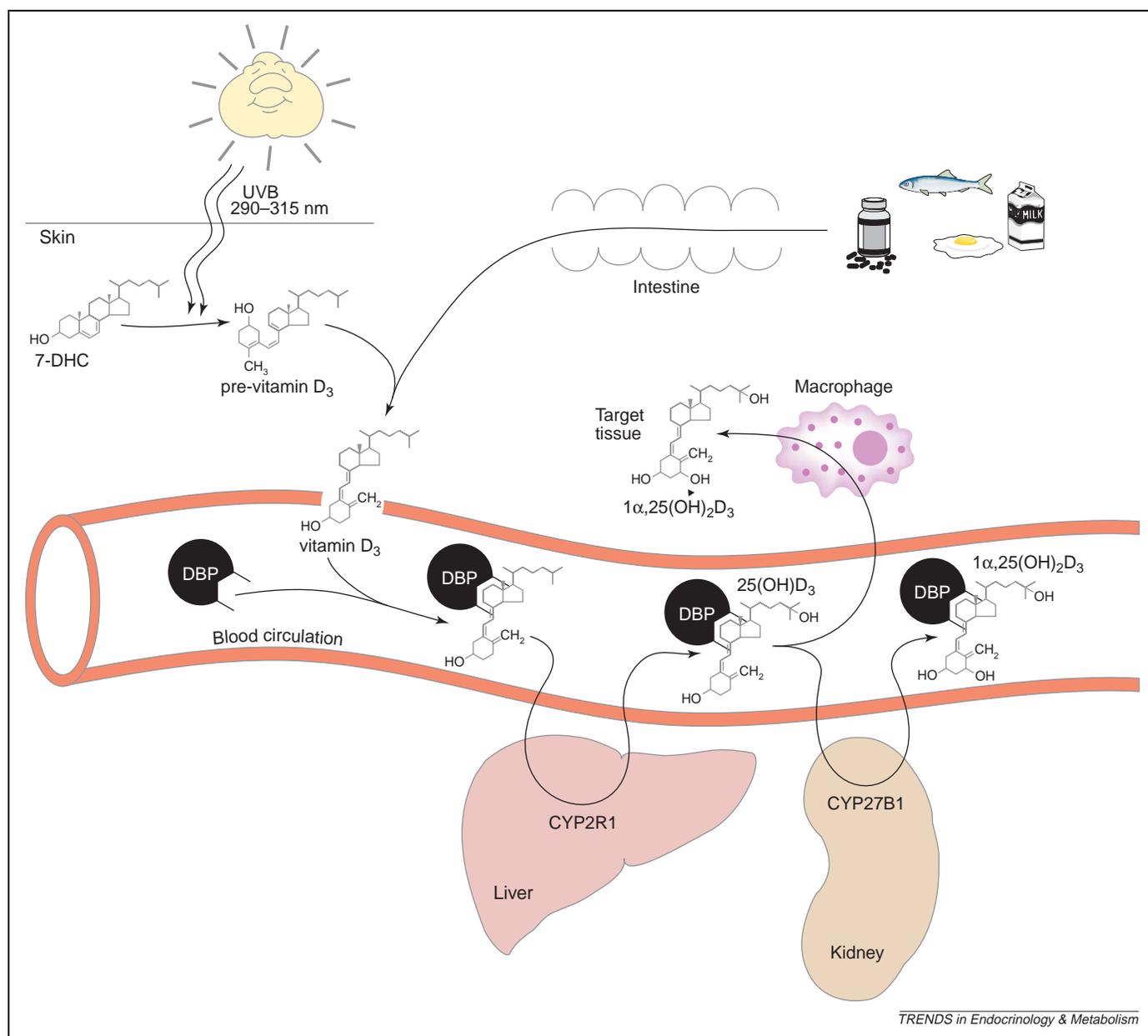
Figure 1. The complex vitamin D system including the regulation of vitamin D serum concentrations, transcellular transport and intracellular metabolism, in addition to its target action. Vitamin D is taken up from food (vitamin D_2 and D_3) or synthesized in the skin [sun light with the photon energy wavelength of 290–315 nm causes photolysis of 7-dehydrocholesterol (7-DHC; provitamin D_3) to pre-vitamin D_3, the immediate precursor in the biosynthetic pathway to vitamin D_3]. In the circulation, all vitamin D metabolites are bound to vitamin D-binding protein (DBP). To become active, vitamin D_3 is hydroxylated first in the liver (25-hydroxylases) and successively in the kidney (1α-hydroxylase) to its final form, $1α,25(OH)_2D_3$. Note that macrophages and other cells are also capable of hydroxylating vitamin D to $1α,25(OH)_2D_3$, thereby facilitating locally regulated tissue concentrations.

Addison's and Graves' disease, and Hashimoto's thyroiditis [10,11]. The *CYP27B1* promoter (-1260) variant C is more often transmitted to offspring with type 1 diabetes. This genotype also occurred significantly more frequently in case–control studies of patients with autoimmune endocrinopathies, where promoter variant C was consistently associated with all four disorders [10].

Polymorphisms of the *VDR*: from association studies to function

Common polymorphisms of the *VDR* gene have been reported to affect the risk of breast, colon [12] and prostate cancer, in addition to bone mineral density and immune disorders, including type 1 diabetes [13,14]. The

association of VDR variants with protection from colon cancer might be related to the recent finding that the VDR acts as an intestinal sensor for toxic bile acids: the VDR has a greater sensitivity for binding lithocholic acid than other nuclear receptors [15].

Steroid hormone nuclear receptors are polymorphic: glucocorticoid receptors affect the neuroendocrine control of glucocorticoid function [16], the androgen receptor is involved in testosterone action [17,18] and hormone replacement therapy (via the estrogen receptor) affects lipid metabolism and E-selectin [19]. In addition to their effects on osteoporosis, *VDR* polymorphisms correlated with muscle strength, fat mass and body weight in healthy premenopausal Swedish women [20] in a population-based

study. A similar correlation with muscle strength had previously been shown for non-obese elderly women [21]. One VDR variant has been characterized for its transcriptional activity [22]: the polymorphic FokI site in the VDR affects its *trans*-activating potential, with the shorter FokI variant of 424 amino acids (absence of the FokI restriction site 'F' abolishes the start codon, and translation starts further downstream) displaying a stronger degree of *trans*-activation than the less active 'f' variant with 427 amino acids. This results in a difference in the modulation of the transcription factor IIB. It is conceivable that, depending on the target tissue, the differences in local concentrations of coactivators and abundance of local promoters could lead to augmented or attenuated *trans*-activation. In addition, the vitamin D-mediated response depends not only on VDR structure, with its polymorphic N-terminus, but also on its DNA binding and ligand affinity. VDR functions as a heterodimer with the retinoic X receptor, which undergoes conformational changes after both DNA and ligand interaction. This process ultimately determines the diversified response to $1\alpha,25(OH)_2D_3$. VDR polymorphisms affect the function of peripheral blood mononuclear cells. Phytohaemagglutinin-stimulated growth inhibition by $1\alpha,25(OH)_2D_3$ was significantly different depending on the FokI status (F: absence of the restriction enzyme site; f: presence): FF homozygotes had the lowest ED_{50} (i.e. the strongest growth inhibition of stimulated mononuclear cells) [23]. This would affect macrophage function in the presence of vitamin D deficiency.

VDR polymorphisms are associated with type 1 diabetes mellitus in Caucasians – with two independent studies from Germany showing an association both in a case–control study and in family-based probands [13,24], in Bangladeshi Indians [14], and in Japanese [25], although such an association was not seen in the Finnish population [26] and in a combined large scale analysis from the UK, Romania and Finland [27]. Whereas initial studies used only the polymorphic FokI, BsmI, ApaI and TaqI variants, recently more polymorphisms were identified with the use of a high resolution single nucleotide polymorphism (SNP) map [28]. Haplotypes rather than individual SNPs have recently been associated with asthma in a Quebec-based family study [29].

Vitamin D and the physiology of β-cell secretion and insulin action

The VDR can be viewed as a master regulator of transcription. VDRs are present in pancreatic β-cells and vitamin D is essential for normal insulin secretion [30]. Islet cell insulin secretion is reduced in vitamin D-deficient animals and can be corrected by vitamin D supplementation [30–32]. Interestingly, an animal model with a mutated VDR has been reported to have impaired insulin secretion [33]. These mice with functionally inactive VDRs have a severely disrupted vitamin D signalling system. They show greatly impaired oral glucose tolerance, expression of the gene encoding insulin and insulin secretion. However, in this model, the background of the mice appears to be crucial, because *Vdr*-knockout mice with a different genetic background

had normal β-cell function [34]. When NOD mice, an animal model for human type 1 diabetes, are rendered vitamin D deficient in early life, impaired glucose tolerance is seen by 100 days of age, with a doubling of diabetes incidence at 200 days [35].

The impact of vitamin D deficiency on β-cell function seen *in vitro* and *in vivo* in animal models has been matched by vitamin D studies in human volunteers undergoing hyperglycaemic clamps [36]. In this study of individuals with different racial origins, 26% of the Caucasians and 54% of the African Americans were vitamin D deficient [defined as $25\text{-}OHD_3$ levels $<20\,\text{ng ml}^{-1}$]. There was a significant negative correlation between plasma glucose values at 60, 90 and 120 min and serum $25\text{-}OHD_3$ levels in those who underwent an oral glucose challenge. Furthermore, β-cell function, as measured by the first and second insulin responses, also correlated negatively with $25\text{-}OHD_3$ levels. This correlation between glucose-induced insulin secretion and vitamin D status was also seen in a study of men aged 70–88, 39% of whom were vitamin D depleted. Their 1-h glucose values and area under a glucose curve negatively correlated with vitamin D concentrations [37]. Therefore, vitamin D insufficiency might contribute to the relative insulin deficiency seen in type 2 diabetes. Vitamin D insufficiency has also been associated with an increased risk for type 2 diabetes, with lower $25\text{-}OHD_3$ concentrations in patients with type 2 diabetes than in controls [38]. In a large cross-sectional survey of Americans performed between 1988 and 1994 there was an inverse association between vitamin D status and diabetes in non-Hispanic whites and in Mexican Americans [39].

Vitamin D and vitamin D analogs and their effects on the immune system in type 1 diabetes

Type 1 diabetes is an autoimmune disease, and the self immune system plays a central role in the destruction of the β cell. The detection of the VDR in almost all cells of the immune system, especially antigen-presenting cells (macrophages and dendritic cells) and activated T cells, led to the investigation of a potential role for $1\alpha,25(OH)_2D_3$ as an immunomodulator [40,41]. Not only are VDRs present in the immune system, but immune cells themselves, in particular activated macrophages and dendritic cells, are able to synthesize and secrete $1\alpha,25(OH)_2D_3$ [42]. These cells possess the tools necessary for the final activating step in the synthesis of $1\alpha,25(OH)_2D_3$ (the enzyme 1α-hydroxylase). The effects of $1\alpha,25(OH)_2D_3$ on the immune system are multiple, but all lead to the generation of tolerance and anergy rather than immune activation [40]. In the presence of $1\alpha,25(OH)_2D_3$, dendritic cells mature in the direction of a tolerogenic cell [43], with less expression of major histocompatibility complex (MHC) class II molecules and adhesion molecules necessary for full T-cell stimulation [44]. In addition, cytokines crucial to the recruitment and activation of T cells are suppressed by $1\alpha,25(OH)_2D_3$. The major cytokine driving the immune system towards T helper type 1 (Th1) development, interleukin 12 (IL-12), is almost completely inhibited in the presence of $1\alpha,25(OH)_2D_3$ through interference with the nuclear

factor κB pathway [45]. Several T-cell cytokines are also direct targets for $1\alpha,25(OH)_2D_3$, leading to inhibition of Th1 cytokines, such as IL-2 and interferon γ (IFN-γ), and stimulation of Th2 cytokines, such as IL-4 [46–48] (Figure 2).

Chronic administration of pharmacological doses of $1\alpha,25(OH)$ reduces the incidence of both insulitis and diabetes in NOD mice [49,50], and reduces diabetes in a model of multiple low dose streptozotocin diabetes [51]. The basis for this protection appears to be mainly a restoration of suppressor cell function in NOD mice [52].

A major obstacle to human application of $1\alpha,25(OH)_2D_3$ is its possible side effect of hypercalcaemia as a result of increased Ca^{2+} resorption from intestine and bone. However, in view of the therapeutic potential, structural analogues have been designed and synthesized, with the aim of achieving a dissociation between calcaemic and immune effects. Several of the most promising analogues have been successfully tested in the NOD mouse [53]. The mechanism of protection against insulitis and diabetes appears to be similar to that of $1\alpha,25(OH)_2D_3$. Effects of the analogues on dendritic cell phenotype, regulatory cell induction and β-cell protection have been described [40,54]. The strongest protection against β-cell destruction by $1\alpha,25(OH)_2D_3$ or its analogues can be seen in situations of chronic administration in primary prevention, with treatment being initiated before autoimmune destruction has started. When administered in NOD mice in situations similar to the human situation, where people at high risk of type 1 diabetes are identified on the basis of already having circulating autoantibodies against the β cell, $1\alpha,25(OH)_2D_3$ or its analogues can only achieve disease prevention when combined with a short course of an anti-T-cell immunosuppressant (e.g. cyclosporine A) [55]. This observation again confirms the *in vitro* observations that the main target cell for $1\alpha,25(OH)_2D_3$ action is the antigen-presenting dendritic cell, whereas its anti-T-cell effects are relatively weak.

In humans, several epidemiological studies provide evidence that vitamin D intake can prevent type 1 diabetes (Box 1).

Conclusion and outlook
Vitamin D, the essential vitamin in Ca^{2+} and bone metabolism, has beneficial effects on β-cell function and normal immunity. Its activated form, $1\alpha,25(OH)_2D_3$, prevents diabetes in NOD mice. Vitamin D insufficiency is a risk factor for autoimmune disease and other disorders. Although optimal supplement dosing with regard to immune and β-cell function is not known,
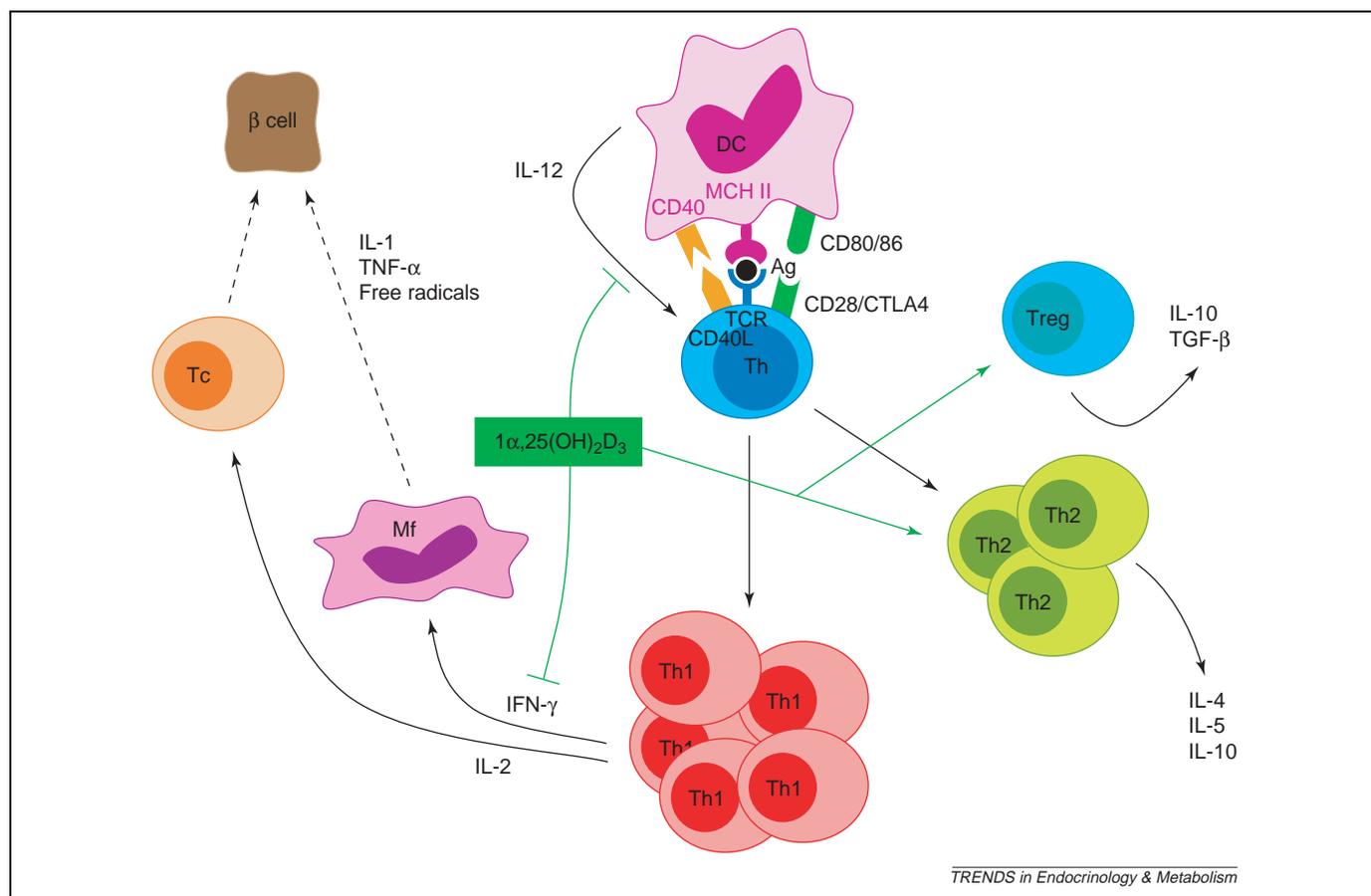

Figure 2. The immunomodulatory effects of $1\alpha,25(OH)_2D_3$. At the level of the antigen-presenting cell (such as dendritic cells; DCs), $1\alpha,25(OH)_2D_3$ inhibits the surface expression of MHC class II-complexed antigen and of co-stimulatory molecules, in addition to production of the cytokine IL-12, thereby indirectly shifting the polarization of T cells from a Th1 towards a Th2 phenotype. In addition, $1\alpha,25(OH)_2D_3$ has immunomodulatory effects directly at the level of the T cell, by inhibiting the production of the Th1 cytokines IL-2 and IFN-γ and stimulating the production of Th2 cytokines. Moreover, $1\alpha,25(OH)_2D_3$ favours the induction of regulatory T cells. Both Th2 and Tregs can inhibit Th1 cells through the production of counteracting or inhibitory cytokines. Together, these immunomodulatory effects of $1\alpha,25(OH)_2D_3$ can lead to the protection of target tissues, such as β cells, in autoimmune diseases and transplantation. CD40L, CD40 ligand; Mf, macrophage; Tc, cytotoxic T cell; TGF-β, transforming growth factor β; Th1, T helper type 1; TNF-α, tumour necrosis factor α; Treg, regulatory T cell.

Box 1. Vitamin D intake and type 1 diabetes prevention

The intake of vitamin D, either as a supplement or via food, has been the subject of recent studies examining populations with a high risk for type 1 diabetes. Hypponen *et al.* found a significantly reduced risk of 0.22 for type 1 diabetes in a birth-cohort study when high-dose vitamin D supplementation (> 50 μg d^{-1}, 2000 U d^{-1}) was given regularly or irregularly [56]. By contrast, those children with suspected rickets during the first year of life had a threefold increased risk of developing type 1 diabetes during later life. Similarly, increased vitamin D intake during pregnancy significantly reduced β-cell autoimmunity in offspring as detected by islet autoantibodies [57]. However, this effect was restricted to vitamin D intake from food. In a Norwegian study, the use of cod liver oil either during pregnancy or in the first year of life was associated with a lower incidence of type 1 diabetes [58]. Whether this was the result of the content of vitamin D or long-chain n-3 fatty acids (or a combination) merits further investigation [59]. Furthermore, a EURODIAB (European Community Concerted Action Programme in Diabetes) subgroup multicentre study of cases and controls found that the risk for type 1 diabetes was significantly reduced in countries with vitamin D supplementation during childhood [60].

substantially higher doses than those currently recommended, possibly as high as 50 μg (2000 U) d^{-}, might be required. A major clinical lesson that can be drawn at this moment is that avoidance of vitamin D deficiency is essential for β-cell function and might contribute to protection against type 1 diabetes in later life. Epidemiological studies have shown that vitamin D deficiency should be avoided in pregnancy, not only because of its effects on bone development, but also because it might increase the incidence of autoimmune diseases, such as type 1 diabetes in genetically at-risk individuals. Exploiting the immunomodulatory effects of $1\alpha,25(OH)_2D_3$ in humans will require the development of safe structural analogues with a dissociation between calcaemic and immune effects. Novel analogues have been developed that are more potent in T cell and dendritic cell modulation and less calcaemic, thus allowing higher doses to target the immune system. These analogues are currently being analysed for their therapeutic potential.

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